WEST Search History

DATE: Thursday, November 20, 2003

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT; PLUR=YES; OP=OR			
L7	L6 and link	35	L7
L6	L5 and operably	138	L6
L5	L4 and inducible	145	L5
L4	L3 and cytomegalovirus	150	L4
L3	L2 and whey	244	L3
L2	L1 and lac	8080	L2
L1	cmv promoter	63261	L1

END OF SEARCH HISTORY

updated search
For promoters.
of 09/747,521
and related cases.

Generate Collection

L7: Entry 1 of 35 File: USPT Oct 14, 2003

DOCUMENT-IDENTIFIER: US 6632436 B2
TITLE: Vaccine compositions and method of modulating immune responses

Detailed Description Text (27):
Opsonins are thought to act as a link or coupling agent between the antigen and the APC to allow more efficient binding, engulfment, and internalization of the antigen. A molecule is defined herein as an opsonin useful in the invention if it binds to a cognate antigen as determined in one or more of the assays of opsonicity described herein. According to the invention, opsonicity is determined in part by detection of binding to an APC and an antigen. For example, fragments of C3 can be bound to sheep red blood cells (SBRC); and opsonins with lectin activity can be directly admixed with microorganisms bearing a cognate carbohydrate.

Detailed Description Text (86):
The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (e.g., in an in vitro transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to includes promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Calif. (1990). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cell and those which direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector may depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion polypeptides or peptides, encoded by nucleic acids as described herein.

Detailed Description Text (87):
The recombinant expression vectors of the invention can be designed for expression of the nucleic acid molecules of the invention in prokaryotic or eukaryotic cells. For example, the polypeptides encoded by the nucleic acid molecules of the invention can be expressed in bacterial cells such as E. coli, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant expression vector can be transcribed and translated in vitro, for example using T7 promoter regulatory sequences and T7 polymerase.

Detailed Description Text (88):
Expression of proteins in prokaryotes is most often carried out in E. coli with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa,

thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D. B. and Johnson, K. S. (1988) Gene 67:31-40), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Detailed Description Text (89):
Examples of suitable inducible non-fusion E. coli expression vectors include pTrc (Amann et al., (1988) Gene 69:301-315) and pET 11d (Studier et al., Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Calif. (1990) 60-89). Target gene expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid trp-lac fusion promoter. Target gene expression from the pET 11d vector relies on transcription from a T7 gn10-lac fusion promoter mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident prophage harboring a T7 gn1 gene under the transcriptional control of the lacUV 5 promoter.

Detailed Description Text (93):
In yet another embodiment, the polypeptides encoded by the nucleic acid molecules of the invention are expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, B., (1987) Nature 329:840) and pMT2PC (Kaufman et al. (1987), EMBO J. 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, adenovirus 2, cytomegalovirus and Simian Virus 40.

Detailed Description Text (94):
In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert et al. (1987) Genes Dev. 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) Adv. Immunol. 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) EMBO J. 8:729-733) and immunoglobulins (Banerji et al. (1983) Cell 33:729-740; Queen and Baltimore (1983) Cell 33:741-748), neuron-specific promoters (e.g., the neurofilament promoter; Byrne and Ruddle (1989) Proc. Natl. Acad. Sci. USA 86:5473-5477), pancreas-specific promoters (Edlund et al. (1985) Science 230:912-916), and mammary gland-specific promoters (e.g., milk whey promoter; U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example the murine hox promoters (Kessel and Gruss (1990) Science 249:374-379) and the--fetoprotein promoter (Campes and Tilghman (1989) Genes Dev. 3:537-546).

Detailed Description Text (109):
A transgenic animal of the invention can be created by introducing nucleic acid molecules encoding the polypeptides of the invention into the male pronuclei of a fertilized oocyte, e.g., by microinjection, and allowing the oocyte to develop in a pseudopregnant female foster animal. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to the transgene to direct expression of a polypeptide of the invention to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Pat. Nos. 4,736,866 and 4,870,009, both by Leder et al., U.S. Pat. No. 4,873,191 by Wagner et al. and in Hogan, B., Manipulating the Mouse Embryo, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of the nucleic acid molecule of the invention, e.g., the transgene in its genome and/or expression of the transgene mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding polypeptides of the invention can further be bred to other transgenic animals carrying other transgenes.

Detailed Description Text (165):
The sequence encoding the alpha chain of murine C3b is amplified by PCR from mouse liver cDNA using an upstream primer corresponding to nt 2301-2324 of Genbank K02782, and a downstream primer complementary to nt 5018-5045. The gene for pneumolysin is

amplified from pneumococcal DNA using an upstream primer corresponding to nucleotides 207-233 of Genbank M17717 and flanked on the 5' end by 12 bases that correspond to nt 5034-5045 of Genbank K02782 and a downstream primer that corresponds to the sequence complementary to nucleotides 599-1622 of M17717 and flanked at the 5' end by a Sph I site. Both products are isolated by agarose gel electrophoresis, eluted using glass beads, and used, in equimolar amounts, in a PCR reaction with excess amounts of the upstream C3b alpha chain primer and the downstream pneumolysin primer. The fusion product is isolated by agarose gel electrophoresis and digested with Sph I. A double-stranded DNA sequence corresponding to the mouse IL-2 secretory sequence (nt 48-107 of Genbank X0 1772) and flanked upstream by a single-stranded Hind III overhang is prepared. The mammalian expression vector pcDNA3 is digested with Hind III and Sph I, and the fragments are ligated into the plasmid so that the CMV promoter is upstream of the IL-2 signal sequence, which is upstream of the C3b alpha chain/pneumolysin fusion gene.

Detailed Description Text (168):
The sequence encoding murine C3 is amplified by PCR from mouse liver cDNA using an upstream primer corresponding to nt 57-78 of Genbank K02782, and a downstream primer complementary to nt 5018-5045. The gene for pneumolysin is amplified from pneumococcal DNA using an upstream primer corresponding to nucleotides 207-233 of Genbank M17717 and flanked on the 5' end by 12 bases that correspond to nt 5034-5045 of Genbank K02782 and a downstream primer that corresponds to the sequence complementary to nucleotides 599-1622 of M17717 and flanked at the 5' end by a Sph I site. Both products are isolated by agarose gel electrophoresis, eluted using glass beads, and used, in equimolar amounts, in a PCR reaction with excess amounts of the upstream C3 primer and the downstream pneumolysin primer. The fusion product is isolated by agarose gel electrophoresis and digested with Sph I. The mammalian expression vector pcDNA3 is digested with Hind III, blunted with Klenow, and then digested with Sph I, and the fusion gene is ligated into the plasmid so that it is downstream of the CMV promoter.

Detailed Description Text (172):
The sequence encoding MBP is amplified by PCR from mouse liver cDNA using an upstream primer corresponding to nt 121-142 of Genbank S42292 and a downstream primer complementary to nt 818-837 and flanked on its 5' side by 12 residues of poly-C. Lysozyme is amplified from chicken embryo cDNA using an upstream primer corresponding to nt 82-102 of Genbank V00428 and flanked on its 5' side by 12 residues of poly-G, which is itself flanked 5' by a sequence corresponding to nt 829-837 of S42292, and a downstream primer complementary to nt 450-468 of V00428 flanked on the 5' side by an Xba I site. Both products are isolated by agarose gel electrophoresis, eluted using glass beads, and used, in equimolar amounts, in a PCR reaction with excess amounts of the upstream MBP primer and the downstream lysozyme primer. The fusion product is isolated by agarose gel electrophoresis and digested with Xba I. The mammalian expression vector pcDNA3 is digested with Hind III, blunted with Klenow, and then digested with Xba I, and the fusion gene is ligated into the plasmid so that it is downstream of the CMV promoter.

Detailed Description Text (175):
The sequence encoding MBP is amplified by PCR from mouse liver cDNA using an upstream primer corresponding to nt 121-142 of Genbank S42292 and a downstream primer complementary to nt 818-837. A double-stranded oligonucleotide is obtained corresponding to nt 196-237 of Genbank V00428 and flanked on its downstream side by a single-stranded Xba I overhang. The pcDNA3 plasmid is digested with Hind III, blunted with Klenow, and digested XbaI. The MBP sequence is ligated to the vector and the product is isolated by agarose gel electrophoresis and glass beads elution. The lysozyme peptide encoding sequence is ligated into the latter product, and, using restriction digest analysis and DNA sequencing, clones are identified in which a single MBP gene in sense orientation is immediately downstream of the promoter and upstream of the lysozyme peptide gene.

Detailed Description Text (224):
The vector pCIL was constructed with a sequence encoding the secretory sequence of murine IL-2. PCI (Promega) is a mammalian expression vector that uses a CMV promoter and an SV40 polyA addition site for constitutive expression of genes cloned downstream of the CMV promoter. A synthetic double-stranded oligonucleotide (GTX 7,8) coding for the mouse IL2 secretory sequence was inserted downstream of this CMV promoter.

Detailed Description Text (227):
To clone HEL into pCIL, the expression vector containing the CMV promoter and the IL2 secretory sequence, the pUC19-HEL was digested with NaeI and a synthetic NheI site was

ligated to the HEL fragment. The NheI-ligated HEL fragment was purified by agarose electrophoresis and ligated to pCIL that had been digested with NheI. Correct orientation was determined by restriction digests. The resulting plasmid, pCIL HEL comprises, progressing downstream, the CMV promoter for mammalian expression, the IL2 secretory sequence coding sequence, and the HEL coding sequence.

Detailed Description Text (229):
pUC19-42K was purified and digested with AvrII and NheI. This yields a DNA fragment coding for the amino terminal 42 amino acids of the C3b.alpha.' chain linked to the GGGGSGGGGS linker. This fragment was purified after electrophoresis through agarose gels and ligated to pCIL HEL that had been digested with NheI. The plasmid was used to transform E.coli and colonies selected for ampicillin resistance. The resulting plasmid pCIL-42K-HEL comprises, progressing downstream: (i) the CMV promoter for efficient expression in mammalian cells; (ii) the IL2 secretory sequence coding sequence; (iii) a sequence encoding the 42 amino terminal amino acids of the C3b.alpha.' chain, which binds to CR1 on APC's of monocytic lineage; (iv) a sequence encoding the GGGGSGGGGS linker; and (v) the HEL coding sequence.

Detailed Description Text (233):
On Day 1 blood serum was collected from each mouse. This "prebleed" represented baseline antibody levels prior to vaccination. 50 microliters (100 micrograms) of DNA in normal saline was then injected into the tibial muscle of each hind leg (100 micrograms per hind leg; 200 micrograms/mouse). Each group of mice received either pCI vector DNA alone, a pCI vector encoding HEL operably linked to the murine IL2 secretory sequence, or pCIL-42K-HEL. On day 14, the injections were repeated. On day 28 blood serum was again collected.

Generate Collection

L7: Entry 9 of 35 File: USPT May 21, 2002

DOCUMENT-IDENTIFIER: US 6391602 B1
**** See image for Certificate of Correction ****
TITLE: MSP-18 protein and nucleic acid molecules and uses therefor

Detailed Description Text (13):
MSP-18 family members can further include at least one domain characteristic of lysyl oxidase, referred to herein as a lysyl oxidase domain or "LOX domain". Lysyl oxidase is an extracellular copper-dependent enzyme that catalyzes the oxidative deamination of peptidyl lysine residues in precursors of various collagens and elastins. The deaminated lysines are then able to form aldehyde cross-links. (Krebs et al. (1993) Biochem. Biophys. Acta. 1202:7-12). The amino acid sequence of lysyl oxidase includes a signal sequence (e.g., amino acids 1 to 21 of human Iysyl oxidase set forth as SEQ ID NO:5, a pro-peptide region (e.g., amino acids 22 to 168 of SEQ ID NO:5), and a region corresponding to the active, processed protein (e.g., amino acids 169-417 of SEQ ID NO:5), which is responsible for the enzymatic function of the molecule. Lysyl oxidase can be further characterized by the presence of a copper-binding site (Krebs et al. (1993) Biochem. Biophys. Acta. 12-2:7-12) having four conserved histidine residues that presumably supply the nitrogen ligands for copper coordination, and a quinone cofactor binding site (Wang et al. (1996) Science 273:1078-1084) (e.g., his289, his292, his294, and his296 of SEQ ID NO:5), also referred to as a "copper talon".

Detailed Description Text (64):
The antisense nucleic acid molecules of the invention are typically administered to a subject or generated in situ such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a MSP-18 protein to thereby inhibit expression of the protein, e.g., by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention include direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, e.g., by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

Detailed Description Text (67):
Alternatively, MSP-18 gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the MSP-18 (e.g., the MSP-18 promoter and/or enhancers) to form triple helical structures that prevent transcription of the MSP-18 gene in target cells. See generally, Helene, C. (1991) Anticancer Drug Des. 6(6):569-84; Helene, C. et al. (1992) Ann. N.Y. Acad. Sci. 660:27-36; and Maher, L. J. (1992) Bioassays 14(12):807-15.

Detailed Description Text (103):
The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (e.g., in an in vitro transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence"

is intended to includes promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Calif. (1990). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cell and those which direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (e.g., MSP-18 proteins, mutant forms of MSP-18 proteins, fusion proteins, etc.).

Detailed Description Text (104):
The recombinant expression vectors of the invention can be designed for expression of MSP-18 proteins in prokaryotic or eukaryotic cells. For example, MSP-18 proteins can be expressed in bacterial cells such as E. coli, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant expression vector can be transcribed and translated in vitro, for example using T7 promoter regulatory sequences and T7 polymerase.

Detailed Description Text (105):
Expression of proteins in prokaryotes is most often carried out in E. coli with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D. B. and Johnson, K. S. (1988) Gene 67:31-40), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Detailed Description Text (107):
Examples of suitable inducible non-fusion E. coli expression vectors include pTrc (Amann etal., (1988) Gene 69:301-315) and pET 11d (Studier etal., Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Calif. (1990) 60-89). Target gene expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid trp-lac fusion promoter. Target gene expression from the pET 11 d vector relies on transcription from a T7 gn10-lac fusion promoter mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident prophage harboring a T7 gn1 gene under the transcriptional control of the lacUV 5 promoter.

Detailed Description Text (111):
In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, B. (1987) Nature 329:840) and pMT2PC (Kaufman et al. (1987) EMBO J. 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook, J., Fritsh, E. F., and Maniatis, T. Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

Detailed Description Text (112):
In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid).

Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert et al. (1987) Genes Dev. 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) Adv. Immunol. 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) EMBO J. 8:729-733) and immunoglobulins (Banerji et al. (1983) Cell 33:729-740; Queen and Baltimore (1983) Cell 33:741-748), neuron-specific promoters (e.g., the neurofilament promoter; Byrne and Ruddle (1989) PNAS 86:5473-5477), pancreas-specific promoters (Edlund et al. (1985) Science 230:912-916), and mammary gland-specific promoters (c.g., milk whey promoter; U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example the murine hox promoters (Kessel and Gruss (1990) Science 249:374-379) and the .alpha.-fetoprotein promoter (Campes and Tilghman (1989) Genes Dev. 3:537-546).

Detailed Description Text (113):
The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to MSP-18 mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub, H. et al., Antisense RNA as a molecular tool for genetic analysis, Reviews--Trends in Genetics, Vol. 1(1) 1986.

Detailed Description Text (120):
A transgenic animal of the invention can be created by introducing a MSP-18-encoding nucleic acid into the male pronuclei of a fertilized oocyte, e.g., by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. The CDNA having the nucleotide sequence depicted in SEQ ID NO:1 can be introduced as a transgene into the genome of a non-human animal. Alternatively, a nonhuman homologue of a human MSP-18 gene, such as a mouse or rat MSP-18 gene, can be used as a transgene. Alternatively, a MSP-18 gene homologue, such as a MSP-18-1 gene can be isolated based on hybridization to the MSP-18 cDNA sequences shown in SEQ ID NO:1 and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to a MSP-18 transgene to direct expression of a MSP-18 protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Pat. Nos. 4,736,866 and 4,870,009, both by Leder et al., U.S. Pat. No. 4,873,191 by Wagner et al. and in Hogan, B., Manipulating the Mouse Embryo, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of a MSP-18 transgene in its genome and/or expression of MSP-18 mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding a MSP-18 protein can further be bred to other transgenic animals carrying other transgenes.

Detailed Description Text (164):
The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for a MSP-18 protein is fused to a gene encoding the DNA binding domain of a known transcription factor (e.g., GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, in vivo, forming a MSP-18-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (e.g., LacZ) which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be

detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene which encodes the protein which interacts with the MSP-18 protein.

Detailed Description Text (263):
To express the MSP-18 gene in COS cells, the pcDNA/Amp vector by Invitrogen Corporation (San Diego, Calif.) is used. This vector contains an SV40 origin of replication, an ampicillin resistance gene, an E. coli replication origin, a CMV promoter followed by a polylinker region, and an SV40 intron and polyadenylation site. A DNA fragment encoding the entire MSP-18 protein and an HA tag (Wilson et al. (1984) Cell 37:767) or a FLAG tag fused in-frame to its 3' end of the fragment is cloned into the polylinker region of the vector, thereby placing the expression of the recombinant protein under the control of the CMV promoter.